MOLECULAR MECHANISMS OF FLOWER DEVELOPMENT: AN ARMCHAIR GUIDE

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Abstract | An afternoon stroll through an English garden reveals the breathtaking beauty and enormous diversity of flowering plants. The extreme variation of flower morphologies, combined with the relative simplicity of floral structures and the wealth of floral mutants available, has made the flower an excellent model for studying developmental cell-fate specification, morphogenesis and tissue patterning. Recent molecular genetic studies have begun to reveal the transcriptional regulatory cascades that control early patterning events during flower formation, the dynamics of the gene-regulatory interactions, and the complex combinatorial mechanisms that create a distinct final floral architecture and form.

CHROMATIN IMMUNOPRECIPITATION
A method to determine the *in vivo* binding of a protein to a DNA sequence.

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Flowering plants represent one of the most successful and diverse groups of organisms on the planet, with more than 250,000 extant species in the wild and thousands more varieties generated by horticulturists through hybridization and other breeding efforts. A challenge for plants is to reproduce successfully in an unpredictable environment, so they determine when conditions are favourable and then generate their gametes *de novo* in the organs of each flower. Gamete transmission through interactions with pollinators has led to the evolutionary diversification of floral forms to maximize reproductive success. Despite the fact that plants such as orchids, roses and snapdragons have very distinctive flowers, most flowers contain just four distinct organ types and their development involves highly conserved molecular mechanisms.

Fifteen years ago, the first genes involved in flower development were cloned from the model plants thale cress, *Arabidopsis thaliana*, and snapdragon, *Antirrhinum majus*. Since then, enormous progress has been made towards understanding various aspects of flower development and defining the molecular genetic pathways that control flower formation[1–3]. The roles of several genes that regulate the spatial and temporal expression of the floral-organ identity genes have been clarified. Furthermore, the discovery of microRNAs (miRNAs) that control several flower-expressed genes has revealed a new level of gene regulation during flower development. Microarray and CHROMATIN IMMUNOPRECIPITATION technologies have been exploited to identify targets of the transcription factors that are involved in floral patterning. These transcription factors function in higher-order complexes, opening new avenues for biochemical investigation. Finally, new genes have been characterized that potentially establish early floral patterns, generate the various cells and tissues of the four floral-organ types and elaborate their final form. The insights gained from these studies are painting an increasingly clear picture of the molecular mechanisms that are involved in flower development. Understanding flower development is also an area of importance for agriculture, with respect to improving traits such as seed yield in grain species, as well as fruit ripening and quality in citrus trees and common garden plants such as strawberry, tomato and pepper.

The basics of flower formation

The development of flowering plants is an orderly progression from the embryo to the mature plant through continuous organ formation from meristems[4]. Most flowering-plant embryos undergo a stereotypical pattern of cell divisions to form a simple structure with

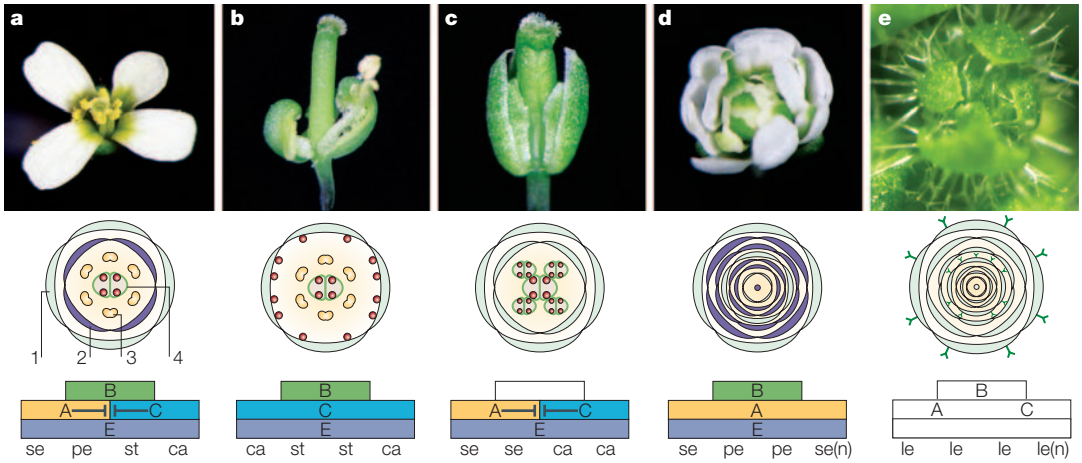


Figure 1 | **The ABCE model for floral-organ patterning. a** | The wild-type *Arabidopsis thaliana* flower consists of four whorls of organs. Sepal (se) identity is conferred in the first whorl by class A activity, petal (pe) identity is conferred in the second whorl by class A and class B activity, stamen (st) identity is conferred in the third whorl by class B and class C activity and carpel (ca) identity is conferred in the fourth whorl by class C activity. Class E activity is required for the specification of each organ type. **b** | An *apetala 2* (*ap2*) flower that consists of carpels in the first whorl, stamens in the second and third whorls, and carpels in the fourth whorl. The mutant lacks class A activity, resulting in expansion of class C activity throughout the flower. **c** | A *pistillata* (*pi*) flower that consists of sepals in the first and second whorls, and carpels in the third and fourth whorls. The mutant lacks class B activity. **d** | An *agamous* (*ag*) flower that consists of sepals in the first whorl, petals in the second and third whorls, and reiterations of this pattern in interior whorls. The mutant lacks class C activity, resulting in expansion of class A activity and loss of floral determinacy. **e** | A mutant flower for four *SEPALLATA* genes (*sep1 sep 2 sep3 sep4*) which consists of reiterating whorls of leaf-like (le) organs. The quadruple mutant lacks class E activity, resulting in impaired class A, B and C function and loss of floral determinacy.

a radial axis and an apical–basal axis[5]. The radial axis establishes the outer epidermal, the underlying cortical and the central provascular tissues. The apical axis determines the shoot apical meristem, which consists of a small collection of stem cells that continuously divide and replenish themselves[6]. Stem cells generate daughter cells that are displaced towards the meristem periphery, where they enter specific differentiation pathways. Only a small number of organs are produced during plant embryogenesis but, on germination, the shoot apical meristem initiates leaf primordia on its flanks in an organized pattern. After a period of vegetative growth, during which the plant maximizes light capture for photosynthesis, a combination of endogenous and environmental cues induce floral development[7]. During this floral transition the shoot apical meristem becomes an INFLORESCENCE MERISTEM, which produces a distinctive architecture of secondary inflorescence meristems followed by meristems for each individual flower.

Most flowers are composed of four distinct organ types that arise in concentric rings, called WHORLS (FIG. 1a). The outer two whorls contain sterile organs that make up the PERIANTH. Sepals arise in the first whorl and form the outer protective covering of the developing bud. Petals arise in the second whorl and are often large and showy, to attract pollinators. In some flowering plants the first and second-whorl organs have the same form and are called TEPALS. The inner two whorls of organs are devoted to reproduction. Stamens arise in the third whorl and produce pollen, which develop into the male gametophytes. Carpels (also known as pistils) arise in the central, fourth whorl and produce OVULES that contain the female gametophytes, the embryo sac. Often the carpels are united or fused together to form the gynoecium. Once fertilized, the gynoecium develops into the fruit harbouring the seeds, although in some species the fruits develop from other parts of the flower, such as the receptacle.

Flower formation occurs through a series of sequential steps. First, FLORAL-MERISTEM fate is specified through the activity of floral-meristem identity genes. Second, the floral meristem is patterned into the whorls of organ primordia through the activity of floral-organ identity genes. Third, the floral-organ identity genes activate downstream effectors that specify the various tissues and cell types that constitute the different floral-organ types. Each of these steps is under strict genetic control, and each involves elaborate networks of positive and negative factors that intersect at various levels to regulate floral morphogenesis.

Induction of floral-meristem fate

The switch from vegetative to reproductive development is controlled by multiple pathways that respond to different environmental and developmental signals[7]. These pathways converge on a set of floral-pathway integrators that activate floral-meristem identity genes such as *LEAFY* (*LFY*) and *APETALA 1* (*AP1*) in *A. thaliana* and their respective homologues *FLORICAULA* (*FLO*) and *SQUAMOSA* (*SQUA*) in *A. majus*. The floral-meristem identity genes ensure that primordia initiated along the periphery of an inflorescence meristem adopt a flower fate. Mutations in these genes result in a partial conversion of flowers into shoot-like structures whereas ectopic expression

INFLORESCENCE MERISTEM
The growing shoot tip during the reproductive, flower-producing phase of development.

WHORL
A concentric ring of floral organs.

PERIANTH
The outermost sterile floral organs that include sepals, petals and tepals.

TEPAL
The organ of a flower perianth in which first and second-whorl organs have the same form.

OVULES
The organs within the carpels that contain the embryo sac and develop into seeds.

FLORAL MERISTEM
A group of cells that is initiated by the inflorescence meristem that generates the organs of a flower.

of these genes is sufficient to convert inflorescence meristems into flowers[8–12]. *LFY* and *AP1* encode transcription factors[13,14] that have partially overlapping roles in specifying a floral-meristem fate, as *lfy ap1* double mutants show a more complete conversion of flowers into shoots than either single mutant[10]. However, to a large degree *AP1* functions downstream of *LFY* (REF. 15), and has been shown to be a direct target of LFY activation[16,17]. Other factors that promote a floral-meristem fate include *CAULIFLOWER* (*CAL*), the function of which completely overlaps with that of *AP1*, and *A. thaliana UNUSUAL FLORAL ORGANS* (*UFO*) or *A. majus FIMBRIATA* (*FIM*).

Functioning in opposition to *LFY* and *AP1*, *A. thaliana TERMINAL FLOWER 1* (*TFL1*) or *A. majus CENTRORADIALIS* (*CEN*) and *AGL24* specify an inflorescence-meristem fate. *LFY* and *AP1* repress *TFL1* and *AGL24* expression in floral meristems, whereas *TFL1* represses *LFY* and *AP1* expression in the inflorescence meristem[15,18,19]. In *tfl1* mutants, *LFY* and *AP1* are ectopically expressed in the inflorescence meristem, causing the normally INDETERMINATE inflorescence to terminate with the production of a flower. Although molecular experiments have shown that *AGL24* is a direct target of AP1 repression and an early target of LFY repression[19], the molecular mechanisms behind the antagonism between *LFY*, *AP1* and *TFL1* remain to be unravelled. Other issues to be addressed include the regulation of *TFL1* by LFY and/or AP1, and the role of TFL1 in *LFY* and *AP1* regulation. The recent identification of a single amino acid within TFL1 that is sufficient to confer its activity as a repressor of flowering should prove useful in identifying potential proteins that interact with TFL1 and better defining the biochemical activity of TFL1 (REF. 20).

The application of DNA microarrays and chromatin immunoprecipitation approaches to the search for LFY targets has proved fruitful in confirming known targets (such as *AP1*) and in identifying new genes that are directly induced by LFY activity[17,21,22]. These new LFY targets include *CAL*, *SEPALLATA* (*SEP*) floral-regulatory genes and several uncharacterized genes that encode putative transcription factors and signalling molecules. The current data indicate that the role of LFY and AP1 in the promotion of floral-meristem identity is dual and includes activation of genes that promote a floral fate and repression of genes that promote an INFLORESCENCE fate. As we gain further insights into the regulatory targets of LFY, the molecular code that distinguishes an inflorescence from a flower will become clearer.

INDETERMINATE
A structure undergoing growth without a defined end.

INFLORESCENCE
A shoot that contains more than one flower.

HOMEOTIC TRANSFORMATION
An event in which an organ of one type assumes the identity of another type within a meristic series.

Floral-organ patterning

Homeotic mutations and the ABC model. A principal function of the floral-meristem identity genes is to activate a small set of genes that specify floral-organ identity. The floral-organ identity genes were originally identified in *A. thaliana* and *A. majus* on the basis of their mutant phenotypes, which featured the HOMEOTIC TRANSFORMATION of one floral-organ type into another. Flowers of *A. thaliana apetala 2* (*ap2*) mutant plants contain carpels in the positions that are normally occupied by sepals, and stamens in the positions that are normally occupied by petals (FIG. 1b). *apetala 3* (*ap3*) and *pistillata* (*pi*) flowers show homeotic transformations of petals into sepals, and stamens into carpels (FIG. 1c), whereas *agamous* (*ag*) flowers form petals in place of stamens and sepals in place of carpels (FIG. 1d). In addition, the floral meristem of *ag* flowers fails to terminate in the production of the fourth-whorl organs. Instead, *ag* flowers are indeterminate and continue to produce new cells that are incorporated into many extra whorls of sepals and petals.

Genetic analysis of these *A. thaliana* floral-homeotic mutants and their counterparts in *A. majus* led in 1991 to the formulation of the classic ABC model for the specification of floral-organ identity[23]. The ABC model postulates that three regulatory gene functions — A, B and C — work in a combinatorial fashion to confer organ identity in each whorl (FIG. 1). A function, conferred by the class A homeotic genes *AP1* and *AP2* in *A. thaliana* and the redundant *AP2*-like genes *LIPLESS 1* and *2* (*LIP1* and *LIP2*) in *A. majus*[24], specifies sepal identity in whorl 1. A function combined with B, conferred by the class B genes *AP3* and *PI* in *A. thaliana* and *DEFICIENS (DEF)* and *GLOBOSA (GLO)* in *A. majus*, specifies petal identity in whorl 2. B function combined with C, conferred by the class C gene *AG* in Arabidopsis and *PLENA (PLE)* and *FARINELLI (FAR)* in *A. majus*, specifies stamen identity in whorl 3. C function alone specifies carpel identity in whorl 4 and also confers floral determinacy. A second key facet of the ABC model is that A function and C function are mutually antagonistic, such that class C activity expands in class A mutant flowers and *vice versa*. Although the details differ, the basic developmental programme for floral-organ patterning that is encapsulated by the ABC model seems to be widely conserved among plant species that have been extensively studied, including tulip[25], petunia[26], primrose[27] and even plants with less showy flowers such as rice[28] and maize[29] (BOX 1).

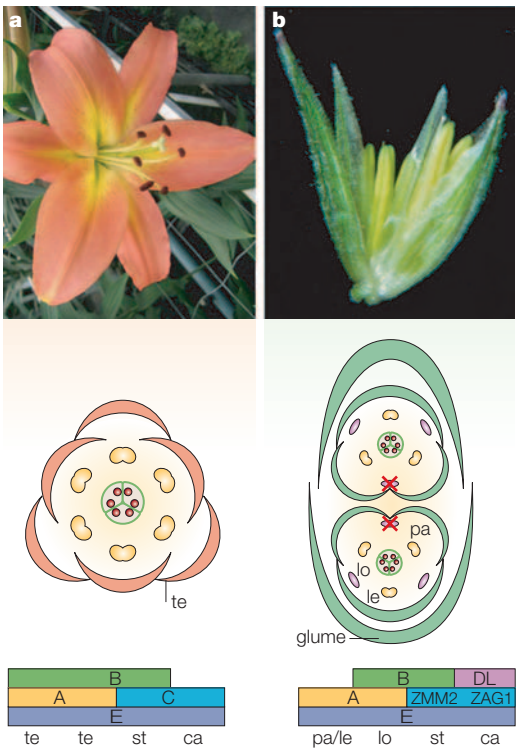
Extending the model: class E genes. Studies of genetic redundancy have led to the identification of the class E floral-homeotic genes. The *A. thaliana* class E *SEP* genes encode related proteins that are redundantly required to specify petals, stamens and carpels, as *sep1 sep2 sep3* triple mutant flowers contain only sepals. *SEP4* is required redundantly with the other three *SEP* genes to confer sepal identity (FIG. 1e), and contributes to the development of the other three organ types[30]. *sep* quadruple mutants show a conversion of all four floral-organ types into leaf-like structures with some carpeloid character, and resemble *ap2 ap3 ag* triple mutants that lack ABC function[31]. *SEP* orthologues have been identified in many divergent plant species. In petunia, the *SEP* orthologues *FBP2* and *FBP5* (*FLORAL BINDING PROTEIN 2* and *5*) specify petal, stamen and carpel identity in a redundant fashion[32], with *FBP2* providing the corresponding E function to *SEP3* in *A. thaliana*[33].

Ovule identity factors. A further class of floral D function genes confers ovule identity on tissues that develop within the carpels[34]. Co-suppression of the petunia *FBP7* and *FBP11* genes causes the replacement of ovules with carpel-like structures, whereas constitutive expression of *FBP11* is sufficient to induce ectopic ovule formation on sepals and petals. In *A. thaliana*, ovule identity is conferred by a clade of four closely related genes: *AG*, *SEEDSTICK* (*STK*), and *SHATTERPROOF 1* and *2* (*SHP1* and *SHP2*) (REF. 35). Ovules are sometimes converted into leaf-like or carpel-like organs in *stk shp1 shp2* triple mutant flowers[34], whereas ectopic expression of either *STK* or *SHP* genes is sufficient to induce the homeotic transformation of sepals into carpeloid organs[36]. The *SEP* genes are also involved in ovule formation, because *sep1*$^{-/+}$ *sep2 sep3* flowers have severely compromised ovule development and resemble *stk shp1 shp2* flowers[36]. Interestingly, genetic experiments have revealed that *STK* and the *SHP* genes have an *AG*-independent role in specifying carpel identity[35,36].

Mechanisms of floral-organ identity gene function. The floral-organ identity genes encode transcription factors. AP2 and LIP1/2 are members of the plant-specific AP2/ERF (ethylene-responsive element binding factor) family of transcription factors. All the other floral-homeotic genes, including the *SEP*, *STK* and *SHP* genes, encode MADS domain transcription factors, which contain a DNA binding domain that is conserved among eukaryotes. A recent phylogenetic analysis identified five subfamilies of MADS domain proteins in *A. thaliana*, with the floral-organ identity proteins falling into the MIKC clade[37]. Ectopic expression of floral-organ identity genes in transgenic *A. thaliana* plants confirmed the main tenets of the ABCE model, and showed that the expected ABCE gene combinations are sufficient to confer floral-organ identity[38,39]. Remarkably, by manipulating the expression of the ABCE genes, flowers with any of the floral-organ types in any of the four whorls can be generated.

The biochemical mechanisms that lead to the control of organ identity are becoming more thoroughly understood. The floral MADS domain proteins

Box 1 | Monocot flowers



The ABC model for flower development describes typical higher EUDICOT flowers that consist of sepals, petals, stamens (st) and carpels (ca). Although MONOCOT flowers contain stamens and carpels, they differ from eudicot flowers in the type of organs that are present in the outer whorls. Liliaceae family members often have two outer whorls of showy petal-like tepal (te) organs (panel a), whereas grass flowers have paleas (pa), lemmas (le) and lodicules (lo) in place of sepals and petals (panel b). A modified ABC model in which B function is present in whorls 1, 2 and 3 has been proposed to explain the presence of tepals in Liliaceae flowers (panel a)[101]. This is supported by the observation of class B *AP3/DEF*-like (*APETALA 3* or *DEFICIENS*-like) and *PI/GLO*-like (*PISTILLATA* or *GLOBOSA*-like) gene expression in the outer three whorls of tulip flowers[25], and the absence or low expression of *AP3/DEF*-like genes in the outermost whorl of other monocots that produce distinct sepals and petals[102].

Studies in maize and rice indicate that class B genes have similar roles in grass and eudicot flowers[29]. Loss of the single *AP3/DEF*-like gene in maize (*SILKY 1*) and rice (*SUPERWOMAN 1*), results in replacement of lodicules by paleas or lemmas, or palea-like organs, respectively, and the replacement of stamens by carpels[28,103]. These homeotic transformations are similar to those observed in *Arabidopsis thaliana* and *Antirrhinum majus* class B mutants, indicating that paleas and lemmas are homologous to sepals, and lodicules are homologous to petals (panel b). Maize contains two potential class C *AG/PLE*-like (*AGAMOUS* or *PLENA*-like) genes (*ZAG1* and *ZMM2*), but redundancy makes it difficult to determine their exact roles[104]. Mutations in *ZAG1* affect floral determinacy but not organ identity. Carpels are still produced in rice plants with reduced expression of the *AG/PLE*-like gene *MADS3* (REF. 105), indicating that other factors are required for carpel specification in grasses. The *YABBY* gene *DROOPING LEAF* (*DL*) is one such factor (panel b), as mutations in *DL* result in complete homeotic transformations of carpels into stamens[28,106]. Although class A *AP1/SQUA*-like (*APETALA 1* or *SQUAMOSA*-like) genes have been identified in maize and rice, their roles in flower development are not well defined.

EUDICOT
A plant that forms two seed leaves during embryogenesis and has three or more pores in its pollen.

MONOCOT
A plant that forms a single seed leaf during embryogenesis.

form dimers to bind to their 'CArG (CC(A/T)$_6$GG) box' DNA target sequence[14]. The class B proteins AP3/DEF and PI/GLO bind DNA only as heterodimers, and both components are required in an autoregulatory feedback loop to maintain transcription from their own promoters[40]. DEF and GLO form ternary complexes with SQUA, an AP1 orthologue, in yeast two-hybrid (Y2H) assays, and these complexes show strongly enhanced DNA binding activity relative to DEF–GLO heterodimers or SQUA–SQUA homodimers[41]. The finding that floral MADS domain proteins can associate in higher-order complexes has led to the formulation of a biochemical QUARTET MODEL[3] (FIG. 2).

Recent studies indicate that the association of floral MADS proteins into higher-order MADS complexes might be the principal mode of combinatorial control for floral-organ specification. Although *sep1 sep2 sep3* triple mutant flowers resemble class BC double mutant flowers, the SEP proteins are not required to activate the expression of the class B and C floral-homeotic genes, as these genes are induced normally in the *sep* triple mutant[42]. Instead, the SEP proteins function as co-factors that provide flower-specific activity to the ABC genes by making complexes of their products. The AP3–PI heterodimer interacts directly with AP1 and SEP3 in co-immunoprecipitation experiments, and indirectly with AG through a SEP3 scaffold[38]. The interaction of class B proteins with class E proteins has also been confirmed in petunia, in which FBP1 and MADS1 form a complex with the SEP3 orthologue FBP2 (REFS 33,43). The *A. thaliana* class C protein AG interacts with SEP1, SEP2 and SEP3 in the Y2H system[44], whereas AP1 and SEP3 interact with each other[45]. A recent comprehensive study of *A. thaliana* MADS protein interactions using a matrix-based Y2H screen[46] has confirmed and extended these findings, setting the stage for *in vivo* analyses of the full complement of floral MADS complexes and the dynamics of the protein–protein interactions within them.

The importance of ternary complex formation to floral-patterning activity became apparent when it was revealed that the floral MADS proteins differ in their ability to activate target gene transcription. The AP3–PI heterodimer binds to CArG-box sequences in the *AP3* promoter, but does not activate transcription. However, the ternary complexes AP3–PI–AP1 and AP3–PI–SEP3 are sufficient to activate the *AP3* promoter, indicating that either AP1 or SEP3 can confer transcriptional activation ability to the AP3–PI heterodimer[38]. SEP1 and SEP2 also function as weak-to-moderate transcriptional activators in luciferase assays[38], but whether this has relevance *in planta* remains to be determined.

Regulation of floral-organ identity genes

In most cases the A, B and C class RNA transcripts are expressed within flowers in spatially restricted patterns that are consistent with their sites of action. mRNAs for the class B and C genes are first detected in stage 3 flowers at the time of sepal initiation and remain present as organ primordia arise and mature

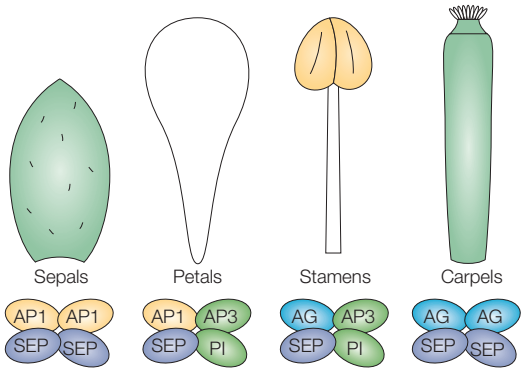


Figure 2 | **The quartet model for potential formation of MADS domain protein-regulatory complexes.** In each whorl, dimers of floral MADS proteins are proposed to bind to CArG (CC(A/T$_6$GG) box binding sites in the promoters of their target genes. These sites could either be adjacent to one another or some distance apart along the DNA. Tetramers form through protein–protein interactions between the MADS protein dimers, which generates a complex that is bound to two CArG-box binding sites. The predicted composition of tetramers in the four whorls are: AP1–AP1–SEP–SEP in whorl 1 to specify sepals; AP1–SEP–AP3–PI in whorl 2 to specify petals; AG–SEP–AP3–PI in whorl 3 to specify stamens; and AG–AG–SEP–SEP in whorl 4 to specify carpels. AG, AGAMOUS; AP1, APETALA 1; AP3, APETALA 3; PI, PISTILLATA; SEP, SEPALLATA.

(FIG. 3). Class E genes have different patterns of expression, with *SEP1* and *SEP2* expressed in all four whorls, whereas *SEP3* and *SEP4* are more spatially restricted. Various regulatory mechanisms control floral-organ identity gene expression. Much of the early work on the spatial and temporal regulation of the A, B and C class genes focused on factors involved in transcriptional activation or repression. However, more recent studies have shown that some of these genes are also regulated at the post-transcriptional level.

The *A. thaliana* floral-meristem identity gene *LFY*, which is expressed throughout young floral meristems, activates different floral-organ identity genes in distinct patterns within the flower (FIG. 3). This seems to result from interactions between the globally expressed *LFY* and cofactors that are expressed in more spatially restricted domains. *LFY* works in combination with *UFO* and *AP1* to activate the class B gene *AP3* in the second and third whorls[13,47], and functions with the meristem gene *WUSCHEL* (*WUS*) to turn on *AG* expression in the inner two whorls[48,49]. In the case of *AG*, this activation might be direct as LFY and WUS bind to sites within an *AG* enhancer element and mutation of these sites results in reduced *AG* expression *in vivo*[49]. Maintenance of high levels of floral-organ identity gene expression during early flower formation requires *ATX1* (also known as *TRITHORAX-LIKE PROTEIN 1*, *TRX1*), a homologue of the *Drosophila melanogaster* histone methyltransferase gene *trithorax*[50]. The plant hormone gibberellin (GA) promotes later expression of the floral-organ identity genes by functioning in opposition to a family of DELLA proteins that repress GA signalling[51].

QUARTET MODEL
This model proposes that tetrameric complexes of MADS proteins determine floral-organ identity in each whorl.

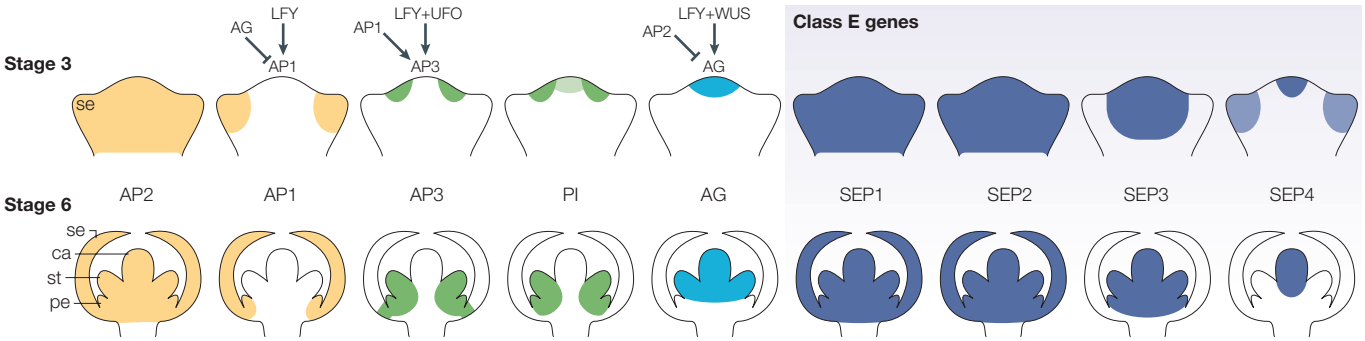


Figure 3 | **mRNA expression patterns of the *Arabidopsis thaliana* floral-organ identity genes during two stages of flower development.** Sepal primordia (se) are present at stage 3, whereas sepal, petal (pe), stamen (st) and carpel (ca) primordia are all present at stage 6. The class A gene *APETALA 2* (*AP2*) is expressed in all four whorls of the flower[125], whereas the other class A gene *APETALA 1* (*AP1*) is expressed specifically in the outer two floral whorls[126]. The class B genes, *APETALA 3* (*AP3*) and *PISTILLATA* (*PI*), are both expressed in cells of stage 3 floral meristems that will form petal and stamen primordia[127,128]. *PI* is expressed at lower levels in the centre of stage 3 floral meristems[127] and *AP3* is expressed at the base of sepals in stage 6 flowers[129]. The class C gene *AGAMOUS* (*AG*) is expressed in the inner two floral whorls[130]. The class E genes *SEP1* and *SEP2* (of the SEPALLATA gene family) are expressed throughout the flower[131,132]. *SEP3* is expressed in the inner three whorls[133], whereas *SEP4* is expressed in the fourth whorl and at low levels in sepal primordia of stage 3 flowers[30]. The interactions that produce these expression patterns are also indicated in the top panel. LFY, LEAFY; UFO, UNUSUAL FLORAL ORGANS; WUS, WUSCHEL.

Antagonism between the A and C class genes. Although floral-meristem identity genes are largely responsible for activation of the ABC class genes, interactions among the floral-organ identity genes themselves influence and refine their expression patterns. For example, expression of the class A gene *AP1* is restricted to the outer two floral whorls at stage 3 as a result of negative regulation by the class C gene *AG* (REF. 52). Likewise *AP2* represses *AG* expression in the outer two whorls[53]. One of the early mysteries within the flower development field was how the globally expressed *AP2* specifically repressed *AG* expression in the outer two whorls of the flower. This now seems to be the result of post-transcriptional regulation of *AP2* by a microRNA. *miR172*, which is expressed at high levels in the inner two floral whorls during later stages of flower development, can cause both cleavage and translational repression of *AP2* (REFS 54–56).

Boundary specification. Besides the A and C class genes, other CADASTRAL genes contribute to the specification of boundaries between the different domains of organ-identity gene activity. LEUNIG (LUG) and SEUSS (SEU) work together as a transcriptional co-repressor complex that represses *AG* expression in the outer two whorls of *A. thaliana* flowers[57]. *STYLOSA* (*STY*), a *LUG* orthologue, has a similar function in *A. majus*[58]. Neither LUG nor SEU has DNA binding activity, indicating that other factors interact with the LUG–SEU complex to regulate *AG* expression. Potential candidates include the AP2-domain containing transcription factors AP2 and AINTEGUMENTA (ANT) (BOX 2); the novel protein STERILE APETALA (SAP); and the homeodomain protein BELLRINGER (BLR) (REFS 59–61). BLR can bind to *AG cis*-regulatory sequences *in vitro* but has not yet been shown to interact with LUG–SEU (REF. 61).

The *A. thaliana* zinc-finger protein SUPERMAN (SUP) functions to maintain the inner boundary of *AP3* expression. Mutations in *SUP* cause an expansion of the *AP3* expression domain and the formation of extra stamens in place of the fourth-whorl carpels[62,63]. Rather than being a direct transcriptional repressor of *AP3* expression, *SUP* has been proposed to regulate the balance of cellular proliferation in the inner two floral whorls[64].

***Post-transcriptional regulation of* AG.** Another level of *AG* regulation was revealed by the analysis of genes identified in two genetic modifier screens. *HUA1* and *HUA2* were isolated in a screen for enhancers of a weak *ag* allele[65]. A *hua1 hua2* double mutant then served as the background for a second enhancer screen that identified several *HEN* (*HUA ENHANCER*) genes. All the *HUA* and *HEN* genes seem to function in RNA metabolism[66]. In *hua1 hua2 hen2* and *hua1 hen2 hen4* mutants, *AG* mature transcript levels are reduced and two larger *AG* transcripts are produced, indicating that these genes have a specific role in *AG* pre-mRNA processing[67]. These longer transcripts result from premature polyadenylation that occurs within the second intron. Currently it is not known whether HUA1, HUA2, HEN2 and HEN4 aid in the production of a full-length mature *AG* mRNA by promoting splicing or inhibiting premature polyadenylation. Two other *HEN* genes, *HEN1*, which encodes an miRNA methyltransferase and *PAUSED/HEN5* (*PSD*), which encodes an exportin-like protein, seem to be important for miRNA biogenesis and tRNA export. Mutations in these genes affect the expression of a number of targets, including *AG* (REFS 68,69).

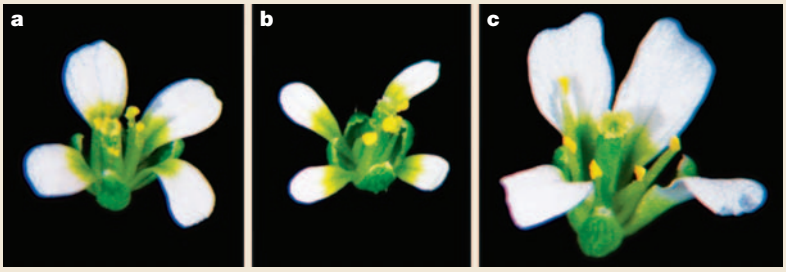
Floral-meristem feedback loop. Temporal regulation of *AG* expression is required for the termination of floral-meristem activity, which occurs through

CADASTRAL
A gene activity that defines the boundaries of a region within a flower.

DIFFERENTIAL DISPLAY SCREEN
A molecular method to identify sequences that are enriched in one genotype or set of conditions compared with another.

a temporal-feedback loop. Following formation of the sepals, petals and stamens, the floral meristem is consumed in the formation of the carpels. During this process the transcription factors LFY and WUS induce the expression of *AG* in the inner two whorls[48,49]. *WUS* is required to maintain the floral meristem in a proliferative, uncommitted state[70], and is expressed in a subset of floral-meristem cells that will form the precursors of the stamens and carpels. *AG* activation leads in turn to the repression of *WUS* transcription[48,49], because *ag* mutant flowers are indeterminate and maintain *WUS* expression in the centre of the flower. Therefore, repression of *WUS* is necessary to terminate meristem activity at the appropriate time to allow the cells in the centre of the flower to differentiate into carpel primordia. ULTRAPETALA 1 (ULT1), a SAND domain putative transcription factor[71], confers at least part of the timing element to this feedback system. *AG* activation is delayed in the centre of *ult1* floral meristems[72] and correlates with a *WUS*-dependent reduction in determinacy in *ult1* flowers[73].

Box 2 | **Regulation of flower size**



Flower size can vary greatly between closely related species. Although the size of an organ reflects the number and size of the cells contained in it, altering cell number or cell size through genetic manipulation is often not sufficient to change the final size of an organ. Compensatory mechanisms seem to function within developing organs to maintain overall organ size[107], but some genes work outside such mechanisms. *ARGOS* (an auxin-regulated gene that is involved in organ size) and *AINTEGUMENTA* (*ANT*) in *Arabidopsis thaliana*, might function in a single pathway that is downstream of the plant hormone auxin[108]. Mutations in either gene result in smaller floral organs (compare a wild-type flower in panel a with an *ant* mutant in panel b), whereas constitutive expression of either gene results in larger floral organs (panel c shows a *35S::ANT* flower)[108–112]. *ARGOS* and *ANT* are thought to promote growth within developing organs by maintaining cells in a division-competent state[112]. Members of the YABBY gene family, including *FILAMENTOUS FLOWER* (*FIL*) and *YABBY 3* (*YAB3*) in *A. thaliana* and *GRAMINIFOLIA* (*GRAM*) in *Antirrhinum majus*, promote abaxial identity and lateral growth of leaves and floral organs. The juxtaposition of cells with adaxial and abaxial identities has been proposed to promote lamina expansion[113]. Mutations in *GRAM* result in smaller leaves and petals[114], whereas *fil yab3* double mutants produce small leaves and radialized floral organs[115]. Furthermore, expression of *YAB3* under the control of an abaxial domain promoter results in the formation of large leaves and floral organs (Y. Eshed, personal communication). Another important regulator of growth is the *A. majus CINCINNATA* (*CIN*) gene, a member of the TCP domain transcription-factor family. Interestingly, although *CIN* promotes petal growth, it restricts growth within developing leaves[116], indicating that different growth mechanisms might operate in leaves and floral organs. A recent QTL analysis of *A. thaliana* leaf and floral-organ size supports this hypothesis: of the eight QTLs identified that affect floral organs, only two also affect leaf formation[117].

Repression of floral-organ identity genes during early development. Finally, during early stages of vegetative development the floral-organ identity genes are globally repressed through the action of several genes including *EMBRYONIC FLOWER 1* (*EMF1*), *EMBRYONIC FLOWER 2* (*EMF2*) and *FERTILIZATION-INDEPENDENT ENDOSPERM* (*FIE*). Mutations in these genes result in premature expression of floral-organ identity genes and the production of flowers and flower-like structures just after germination. Other genes such as *CURLY LEAF* (*CLF*), *INCURVATA 2* (*ICU2*) and *MULTICOPY SUPRESSOR OF IRA1* (*MSI1*) also function during vegetative development to maintain patterns of homeotic gene repression[74–76]. FIE, EMF2 and CLF can interact to form a Polycomb group (PcG) protein complex that is similar to the Polycomb repressive complex 2 (PRC2) of animals[77]. PRC2 can modify chromatin structure through its histone methyltransferase activity[78]. It is now clear that the floral-organ identity genes are subject to complex regulatory networks. Strict spatial and temporal control of these genes might be a consequence of the reduced fitness that can result from alterations in floral-organ identity gene expression.

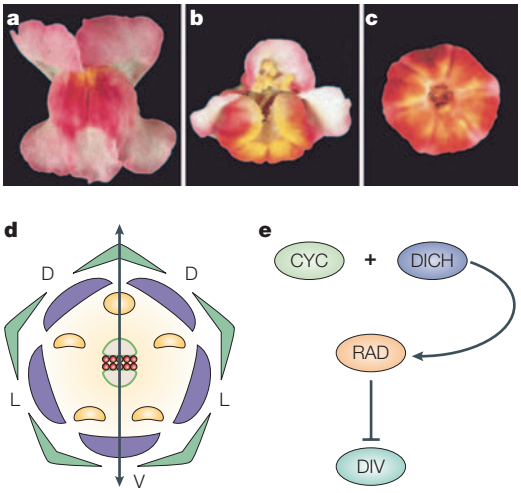
Targets of floral-organ identity gene activity

The past few years have seen a rapid acceleration in the discovery of downstream genes in the floral-organ identity gene-regulatory hierarchies. The first target of a floral MADS protein that was not itself a MADS locus was *NAC-LIKE, ACTIVATED BY AP3/PI* (*NAP*), identified in a DIFFERENTIAL DISPLAY SCREEN for genes that are directly regulated by *AP3* and *PI* (REF. 79). *NAP*, which encodes a member of the plant-specific NAC transcription-factor family, is induced by AP3–PI and controls the transition from cell division to elongation in developing petals and stamens. Maintaining *SUP* expression at the boundary between the stamen and carpel whorls requires *AP3*, *PI* and *AG* (REF. 80), although it is unclear whether this regulation is direct or indirect. A large-scale microarray assay uncovered targets of *AP3* and *PI* in petals, stamens, or both tissues together[81]. A total of 47 genes were recovered, 11 of which are expressed predominantly or exclusively in flowers.

Several other microarray experiments have found direct targets of *AG* regulation in the reproductive organs. *SPOROCYTELESS* (*SPL*), also known as *NOZZLE*, was shown to be a direct target of *AG* activation in a microarray and *in vitro* binding study that was carried out with an inducible *AG* construct[82]. AG binds to a CArG box in the 3′ region of the *SPL* gene, which encodes a transcription factor that regulates ovule patterning and early microsporogenesis. A second study, using an inducible *AG* construct to drive synchronized stamen and carpel formation, recovered 149 genes, including most of the genes known to function in stamen and/or carpel development[83]. Chromatin immunoprecipitation experiments showed that ten of these genes are bound *in vivo* by AG. Among these are *AP3*, *AG*, *SEP3* and two genes that are involved in biosynthesis of GA, which among other activities regulates

Box 3 | **Floral symmetry**

The flowers of many plant species show bilateral symmetry, which is thought to have evolved independently from a radially symmetrical condition (for example, *Arabidopsis thaliana*) as a pollination strategy. The first indication that floral symmetry is under genetic control came from Carl Linnaeus, who described a naturally occurring mutant of toadflax *(Linaria vulgaris)* that altered the bilaterally symmetrical flower to a radially symmetrical form[118]. This abnormal radially symmetrical state was dubbed 'peloria' by Linnaeus, from the Greek word for monster. Peloric flowers are also found in many orchid species and in *Antirrhinum majus*, allowing dissection of the molecular mechanisms of floral-symmetry specification. *Antirrhinum majus* flowers show a bilateral symmetry that is readily seen in the second whorl, which consists of two dorsal petals (D), two lateral petals (L) and a smaller ventral petal (V) (panels a and d). The *DIVARICATA (DIV)* gene encodes a MYELOBLASTOSIS (MYB) domain transcription factor that promotes ventral identity throughout the flower[119]. In the ventral petal, *DIV*, in association with *DEFICIENS* (*DEF*) and *GLOBOSA* (*GLO*), promotes the expression of the *MYBML1* gene, which induces the differentiation of three specialized epidermal cell types[120]. *CYCLOIDEA (CYC)* and *DICHOTOMA (DICH)* encode related TCP domain family transcription factors that have overlapping functions in specifying dorsal identity[121,122]. *cyc* or *dich* mutant flowers show partial ventralization (panel b), whereas *cyc dich* double mutant flowers are completely ventralized and have a radially symmetrical appearance (panel c) resembling that of the peloric toadflax. Expression of *CYC* and *DICH* in the dorsal domain activates *RADIALIS (RAD)*, which encodes a DIV-related transcription factor that contains a single MYB domain[123]. The RAD protein antagonizes DIV protein function in dorsal cells and limits its activity to the lateral and ventral domains (panel e), probably by competing for binding to either DNA target sequences or interaction partners. Remarkably, the peloric toadflax flower is caused by inactivation of the *Linaria cycloidea* gene through DNA methylation[124].



cellular differentiation in anthers[84]. The coordinated regulation of *AP3*, *AG* and *SEP3* is consistent with the quartet model, which predicts that these proteins are present in the same complex.

A global analysis that compared the expression profiles of the floral-homeotic mutants *ap1*, *ap2*, *ap3*, *pi* and *ag* was carried out to identify genes that are expressed predominantly or exclusively in one type of floral organ[85]. This work identified fewer than two-dozen genes that are potentially expressed specifically in sepals or in petals, but more than 1,100 putative stamen-specific genes and 260 putative carpel-specific genes. The study reveals that the reproductive tissues contain a much larger complement of organ-restricted transcripts than the sterile perianth organs. Although formally downstream of the floral-organ identity genes, many of these spatially restricted flower loci lack a CArG box for the MADS protein binding, indicating that they are indirectly regulated by the floral-homeotic gene products.

Novel genes involved in flower formation

Early flower-patterning events remain largely uncharacterized at the molecular level. Mutants with altered organ number and/or organ positioning have been identified, but these phenotypes seem to result from changes in floral-meristem size rather than specific patterning defects. Recently, several genes that are involved in second-whorl organ primordia formation have been identified. *UFO* was found to be a key regulator of second-whorl organ development with the identification of a class of weak *ufo* alleles in which petals are missing or replaced by staminoid petals or filaments[86]. *petal loss* (*ptl*) mutants possess similar defects, but also exhibit alterations in petal orientation and fusion between the first-whorl sepals[87]. Less severe second-whorl defects are observed in *rabbit ears* (*rbe*) and *roxy1* mutants; some petals are present in these flowers, but they are reduced in size and/or altered in morphology[88,89]. All four of these genes function in a whorl-specific rather than an organ-specific manner. Although the *ufo* phenotype is due to ectopic second-whorl *AG* activity, the defects in *roxy1* and *ptl* flowers cannot be rescued by loss of *AG* function[86,87,89]. Therefore, it seems that at least two different pathways regulate second-whorl organ initiation. *PTL* is expressed in the first-whorl sepals, but not in second-whorl cells, indicating that the second-whorl defect might be an indirect effect of overgrowth in the first whorl[90]. ROXY1, a glutaredoxin, might function as a post-translational modifier of proteins that is involved in petal initiation and growth.

Floral-organ architecture can vary widely between individual plant species. One aspect of this morphological diversity is the degree of fusion between the floral parts. Floral organs can grow as separate entities or as fused structures. For example, four separate petals are found in the second whorl of *A. thaliana*

flowers, whereas five fused petals create the corolla tube of a petunia flower. Members of the NAC transcription-factor family are required for establishing boundaries between lateral organ primordia that result in organ separation. Inactivation of petunia *NO APICAL MERISTEM* (*NAM*); *A. thaliana CUP-SHAPED COTYLEDON 1* and *2* (*CUC1* and *CUC2*); and *A. majus CUPULIFORMIS* (*CUP*) results in fusion of cotyledons, leaves and/or floral organs[91–94]. Evidence that these proteins function as repressors of cell division comes from the recent demonstration that CUP interacts with a TCP domain transcription factor[94]. Several members of the TCP domain protein family (BOX 3) are known to regulate cell proliferation during organ development[95].

NAM, *CUC1*, *CUC2*, *CUP* and several other members of the NAC gene family share a binding site for three members of the *MIR164* family of miRNAs[96]. Expression of *miR164*-resistant forms of *CUC1* and *CUC2* demonstrates the importance of their miRNA regulation, and *miR164* has been shown to direct the degradation of the *CUC* mRNAs[56,97–99]. *CUC* transcript levels are reduced in plants that overexpress *miR164* (REFS 56,98). Rather than restricting the expression of *CUC1* and *CUC2* to the boundary domain, *miR164* might regulate the overall abundance of the *CUC* transcripts. This is supported by the overlap in the expression domains of *miR164c* and the *CUC* genes, as well as by the increased levels of *CUC1* and *CUC2* mRNA in *miR164c* loss-of-function mutants[97].

Conclusions and future horizons

The past 15 years has seen an extraordinary amount of progress in the field of flower development. Research has advanced from the cloning of the first floral-homeotic genes towards understanding the combinatorial control of floral-organ identity at the molecular and biochemical levels. We are developing a picture of the regulatory hierarchies that function at the transcriptional and post-transcriptional level, and we are also identifying components of genetic pathways that specify the pattern, morphology and structure of flowers and their component organs.

The prospects for addressing the outstanding questions in flower biology are bright. Future investigations will doubtless continue to improve our limited knowledge of the complex regulation of the floral-organ identity genes, and to enhance our understanding of the composition and *in planta* function of higher-order MADS protein complexes. Other important lines of inquiry will be to link the regulatory activities at the top of the floral-organ patterning regulatory hierarchy with the downstream events that lead to terminal differentiation of tissue types, and to determine how the targets of the homeotic genes sculpt the different floral organs. We are now poised to move beyond cataloguing the floral-organ transcriptome and the MADS protein target genes towards distinguishing direct from indirect regulatory interactions and characterizing the biological functions of the full effector-gene complement. An even more ambitious task will be to integrate the various pathways that specify floral-organ patterning, number, size, shape and symmetry into a comprehensive system. Computer modelling studies can be increasingly used to generate and test predictions about the behaviour of biological systems. Finally, the current research highlights the synergy obtained by simultaneously analysing several genetically tractable flowering plant species rather than relying on a single model organism. Broad-scale comparative studies, such as the evolutionary genomics effort of the Floral Genome Project[100], will be extremely valuable to probe the origin of flowers, and to dissect the factors that produce and control their remarkable morphological diversity.

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Competing interests statement

The authors declare no competing financial interests.

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